In vitro imaging of single living human umbilical vein endothelial cells with a clinical 3.0T MRI scanner

Z. Zhang[1], E. van den Bos[2], M. de Jong-Popijus[2], M. Bernsen[1], D. Duncker[2], G. P. Krestin[1], P. A. Wielopolski[1]
[1]Radiology, Erasmus MC, Rotterdam, Netherlands, [2]Experimental Cardiology, Erasmus MC, Rotterdam, Netherlands

Introduction: Present vasculogenic therapies explore the usage of transplanted endothelial cells (ECs) and endothelial precursor cells (EPCs) to accelerate vasculogenesis. The possibility for a non-invasive mean to monitor the transplanted cells to follow their bio-distribution and biological function would be appropriate. To monitor cells using MRI, cells could be labelled, e.g., using superparamagnetic iron oxide particles (SPIOs). SPIOs accumulated in cell vacuoles can provide a signature in the form of a signal void from the presence of intracellular iron. However, to understand the in vivo behaviour of transplanted cells, it is necessary to study living and migrating cells in vitro. The aim of the present study is to image SPIO-labeled living single cells over time.

Materials and methods

Cell culture and SPIO labelling

HUVEC were labelled using Endorem (Guerbet S.A., Paris, France) and Lipofectamine 2000 (Invitrogen, Breda, The Netherlands). The iron concentrations used for labelling were 10 µg/mL, 20 µg/mL, 40 µg/mL and 80 µg/mL, respectively, for 24 hours. For controls, cells cultured in medium were used. A subset was replated and grown for 5 days. Intracellular uptake of iron was verified by Prussian blue staining and electron microscopy. Toxicity of intra-cellular iron was studied by determining cell death, mitochondrial membrane potential and proliferative capacity of labelled and unlabeled cells. Proliferative capacity of labelled v.s unlabeled cells was determined by plating $5x10^3$ cells in standard culture dishes, followed by counting of the number of cells on days 1, 3, 5 and 10 after labeling using a hemacytometer.

MRI

Labeled HUVECs were seeded into 4-well culture dishes with endothelial growth medium (EGM) without Gd-DTPA and incubated. Cells were cultured for 12 hours before the first MRI scan. Before every MRI, the medium was replaced with fresh EGM with Gd-DTPA (200:1) to provide better signal-to-noise (SNR) and contrast-to-noise (CNR) ratios for the MRI scanning protocol; then cells were observed by light microscopy. A single loop solenoid coil with an inner diameter 1.0 cm was used for signal reception. MR imaging was performed for 5 consecutive days. Scanning was performed on a 3.0T clinical MR scanner (General Electric Medical Systems, Milwaukee, USA) using a T1-weighted 3D spoiled gradient recalled (SPGR) sequence, scan time ~ 15 min, voxel size of 39 × 48 × 100 μm^3.

Results

At all concentrations tested a labelling efficiency of 100% was obtained. Retention of SPIO label by HUVECs was determined after 5 days of cell culturing by magnetic cell sorting. As shown in Figure 1 significant loss of label occurs since only 1.3% to 60% of the cells could be magnetically isolated, depending on the initial concentration used for labelling. Labelling concentrations of ≥ 20 µg/ml resulted in a decreased mitochondrial membrane potential. For a concentration of 80 µg/ml, a slight but significant increase in cell death was observed ($p < 0.05$, n=3). Based on former results and observation that a labelling concentration for SPIO of 20 µg/ml also did not result in any reduction of the proliferative capacity of labelled HUVECs, we chose this concentration for MRI of living HUVECs. For this concentration, the intra-cellular iron content after 24 hrs of labelling was 9 pg/cell and 2 pg/cell after 5 days. Single living cells could be detected in vitro using MRI with each signal void corresponding to the position of a labeled cell as determined normal light microscopy. Figure 2 shows one slice from a 3-dimensional data set obtained by imaging a dish containing living labeled HUVECs (20 µg/mL for 24h labelled, day 1, day 5) in culture medium with Gd-DTPA (200:1). Some cell groups lost their label as shown in Figure 2.

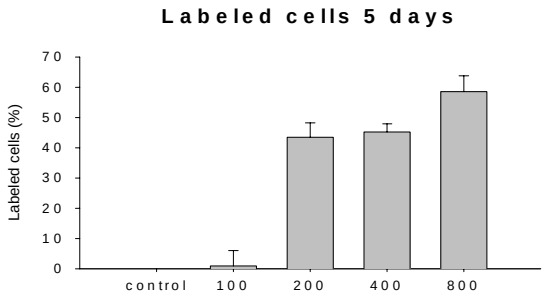

Figure 1: Retention of SPIO particles in HUVECs. HUVECs were labeled with 100, 200, 400, or 800 µg SPIO using Lipofectamine in 10 ml medium (horizontal axis). Control HUVECs were incubated in medium only. Labeled cells and control cells were then cultured for 5 days (37^0c; 5% CO_2). On day 5 the percentage of cells still contained SPIO was determined by Magnetic Activated Cell Sorting (vertical axis). After 24 hrs all cells were labeled with SPIO at all concentrations tested, 100% cells labeled (n=3). After 5 days different percentages of the total cell population had lost SPIO content, depending on the concentration used for labeling (n=3).

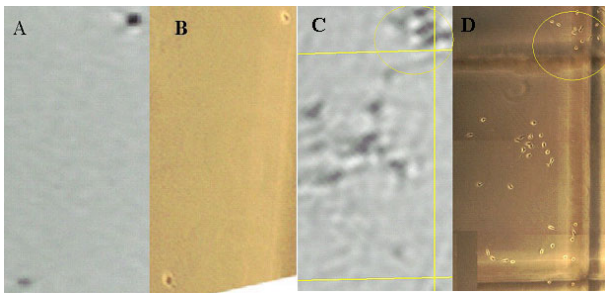

Figure 2: Comparison between MRI and optical microscopy. Panel A shows a slice from a scout image obtained in 14.53 minutes scan time. Signal voids correspond to cells as shown in the corresponding normal light microscopy field (panel B) after labeled 1 day. Panel C shows after 4 days the same cell (up on A, B) divided into a group of cells and Signal voids less than them as shown in the corresponding normal light microscopy field (panel D). A few of cells were shown on normal light microscopy field, but they were not shown the same place on MRI. Imaging parameters: TR=37.2 ms, TE=10.54 ms, flip angle=50^0, FOV = 2×2 cm^2, Matrix = 512×416, NEX = 1.0, scan time = 14.53 minutes. Resolution = 39 × 48 ×100 μm^3.

Discussion/Conclusions

A high-resolution protocol was used on a clinical 3.0T MRI scanner with a standard gradient imaging set. All cells were visible with T2* weighted images as dark spots, some darker and larger than others (owing to both partial volume effects inherent to the imaging parameters and probably differential amounts of label incorporated into each cell). The number of cells as seen on microscopy images were compared with the number of signal voids on MR imaging with a perfect correlation between both. Furthermore, intracellular iron concentrations after 5 days were lower than could be expected based upon cell proliferation. This implies iron was released from intracellular stores. This could further contribute to the loss of iron label and detectability especially in the number of cells that already have low intracellular stores. The mechanism by which cells release their iron is unclear. It could be possible that it occurs during mitosis. Future studies should be aimed at investigating this possibility, since the present study was not specifically designed for this purpose. Loss of label detectability is a limiting factor in single cell tracking studies and, to our knowledge, has not been studied and described before. It is a warning for future in vitro and in vivo studies that single cell tracking over periods longer than 5 days could be difficult. Iron distribution and retention should therefore be assessed for each cell type used since differences in cell biology, doubling time and initial intracellular iron uptake could markedly differ and influence detectability over time.